PERMEABILITY OF THE CELL WALLS OF ALLIUM

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Many investigators have reported that the tissues of higher plants are almost if not quite impermeable to inorganic salts. They have usually attributed this phenomenon to the impermeability of the protoplasm to the salts used. It is quite probable, however, that the cell walls themselves may exert an important influence on the permeability of tissues. It is of interest, therefore, to point out a striking example of the impermeability of the cell wall, which was found when it was attempted to investigate, by the diffusion method recently described by the writer,[1] the permeability of epidermis from the inner surface of bulb scales of the onion. The principle of the experiment and the apparatus used were the same as in the writer's experiments on *Laminaria*, as recorded in the paper cited. Certain modifications were necessary, however.

In order to avoid injury due to drying out of the epidermis (which consists of a single layer of cells), it was necessary to reduce as much as possible the time intervening between the act of stripping the epidermis from the scale and that of filling the cells with solutions. The whole operation usually occupied about 30 seconds, a time which caused no observable injury to the cells.

Dead material was prepared by exposing freshly removed sheets of tissue to chloroform vapor for a period of one hour, then immediately immersing them in a large volume of distilled water, which was several times renewed. After 15 days in distilled water the dead tissue was used in the usual manner.

The salt solutions used in the lower cells were always 0.05 M, a concentration hypotonic to the living cells of the onion epidermis. In the upper cells there was placed distilled water having a specific conductivity of about 2×10^{-6} mhos. Extreme precautions were used to prevent access of dust, acid vapors, or any other soluble material to the distilled water in the upper cells. In all the

[1] Brooks, S. C., A new method of studying permeability. Bot. Gaz. 64:306–317. *figs.* 2. 1917.

experiments on this tissue the distilled water was obtained by distillation from an apparatus made entirely of glass, and which had been in constant operation for several weeks prior to the collection of the sample here used.[2] All the kations were used in the form of chlorides, thus making it possible to determine their concentration in the upper cell by two entirely independent methods. The conductance of known concentrations from 10^{-7} M to 10^{-3} M of the salts used was determined and a curve plotted showing for each salt the concentrations corresponding to any given conductance. The concentration of a given salt diffusing into the distilled water in the upper cell was then ascertained by comparison of the conductance of the solution in the upper cell with the curve for the corresponding salt. In addition, the chlorides in the upper cell were determined nephelometrically by the method of RICHARDS and WELLS.[3]

In neither living nor dead tissues could the presence of chlorides in the upper cell in excess of 3×10^{-5} M be detected nephelometrically, even during experiments whose duration exceeded 24 hours. The changes in conductivity were also such as would indicate a negligible increase in the concentration. It seems therefore that little or no salt can pass through the epidermis.

Experiments were then tried to determine the permeability of the tissue to dyes. The diffusion of Bordeaux red through the diaphragm from an 0.1 per cent aqueous solution in the lower cell into distilled water in the upper during 96 hours was insufficient to cause any visible change in the color of the distilled water. A similar experiment, in which the lower cell contained a 1 per cent aqueous solution of eosin (Merck's eosin bluish), was continued for 7 days; at the end of that time the distilled water in the upper cell could not be distinguished in color from fresh distilled water, even by the use of a colorimeter.

The experiments on dyes (as well as those on acids and alkalies, subsequently described) were performed on dead tissue.

[2] Water distilled from glass becomes better the longer distillation is continued, since the constant exposure to steam and hot water soon removes the more soluble constituents of the glass. Water such as that here used may be regarded as having no appreciable toxicity.

[3] RICHARDS, T. W., and WELLS, R. C., The nephelometer, an instrument for detecting and measuring opalescent precipitates. Amer. Chem. Jour. 31:235. 1904.

In the use of indicators we possess an extremely sensitive and reliable means of demonstrating the presence of small amounts of free acid or alkali in a solution. It would be possible therefore to detect the diffusion through the diaphragm of tissue of small amounts of hydrochloric acid or sodium hydroxide by adding a small amount of a suitable indicator to the distilled water in the upper cell of the apparatus. In the lower cells 0.1 M solutions of the acid and alkali were used.

A period of 43 hours was insufficient to allow the passage of an amount of sodium hydroxide great enough to cause any change in the color of the distilled water containing about 0.01 per cent of phenolphthalein, as determined by comparison in a colorimeter with fresh distilled water. The change of hydroxyl-ion concentration necessary to cause the first visible change in the color of the phenolphthalein would be that from 1×10^{-9} M to 1×10^{-5} M.

The turning point of Congo red lies at a hydrogen-ion concentration of 1×10^{-4} M. An increase of less than 1×10^{-4} M in the hydrogen-ion concentration of distilled water containing Congo red will then cause the appearance of the blue coloration in the indicator. Experiments were conducted in which the lower cells were filled with 0.1 M hydrochloric acid, and the upper cells with distilled water containing barely sufficient Congo red to cause a distinct red coloration; these showed that a period of 3–5 hours was sufficient to cause the color change in the indicator. Control experiments in which the lower cell was filled with pure distilled water showed no color change in the upper cells during 19 hours.

In order to eliminate the possibility that the permeability to hydrogen ions was the result of the action of the 0.1 M hydrochloric acid on the tissue, several of the cells in which there had been a diffusion of acid were simply rinsed out thoroughly, and the lower cell finally filled (after preliminary rinsing with the solution) with 0.1 M sodium hydroxide. The upper cell was filled with distilled water containing a slight amount of phenolphthalein. There was no color change in the distilled water up to the end of the experiment, a period of 3 days.

The inner epidermis of onion bulb scales, at least when its cells are dead, is therefore but slightly permeable to hydrochloric acid, and not perceptibly so to any other of the substances tried. These

included sodium, calcium, and aluminium chlorides, Bordeaux red, eosin, and sodium hydroxide.

This extraordinary impermeability is confined to the exterior cell walls of the epidermis, as will be seen by the following simple experiment. A sheet of epidermis stripped from the scale and mounted in water on an ordinary microscope slide, then irrigated with a 0.4 M sodium chloride solution, was strongly plasmolyzed within 30 seconds. In order that plasmolysis should occur, it was necessary that the plasmolyzing solute should pass into the space between the cell wall and the retracted protoplast. Some part of the cell wall is therefore freely permeable to sodium chloride.

Pieces of the scale, about 2 cm. square, with the epidermis still in place, were then placed in a 0.4 M sodium chloride solution. At intervals up to 30 minutes pieces were withdrawn, the surface dried with filter paper, and a small piece of epidermis from near the center of the piece of scale removed. These were placed between a microscope slide and cover slip, no water being added, and in all cases their cells were found to be wholly normal in appearance; but a few seconds' irrigation with an 0.4 M. sodium chloride solution now sufficed to cause violent plasmolysis. These experiments show that the exterior walls of the epidermal cells form a continuous layer highly impermeable to most substances and comparable to certain seed coats as described by previous investigators.[4]

Summary

1. The exterior cell wall of the epidermis from the inner surface of onion bulb scales is slightly permeable to hydrochloric acid, while it is practically impermeable to various salts, dyes, and to sodium hydroxide.

2. It is necessary to consider the influence of impermeable cell walls in interpreting experiments on the permeability of plant tissues.

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[4] Cf. BROWN, A., The selective permeability of the coverings of the seeds of *Hordeum vulgare*. Proc. Roy. Soc. London, B **81**:82. 1909; SCHROEDER, H., Über die selektive permeable Hülle des Weizenkornes. Flora **102**:186. 1911; SHULL, C. A., Semipermeability of seed coats. BOT. GAZ. **56**:169–199. 1913.